A Phase 2 safety, dose-finding and efficacy study evaluating viscoelastic testing (VET) guided tissue plasminogen activator (tPA) treatment in critically-ill patients with pro-thrombotic acute respiratory failure (ARF) .

'VETtiPAT ARF'

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Locations of Study:
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Bankstown Hospital Intensive Care Unit

1.0 SUMMARY

Study Title	A Phase 2 safety, dose-finding and efficacy study evaluating viscoelastic testing (VET) guided tissue plasminogen activator (tPA) treatment in critically-ill patients with pro-thrombotic acute respiratory failure (ARF) .
Aims/Objectives	To determine whether the administration of alteplase normalises fibrinolysis in patients with ARF and a hypercoagulant and hypofibrinolytic disease phenotype, thus at high risk of thrombosis. *Stage 1:* establish the safety and dose range of VET-guided alteplase treatment in ARF patients at high risk of thrombosis. *Stage 2:* determine whether the administration of alteplase to ARF patients at high risk of thrombosis normalises fibrinolysis and improves organ function.
Study design	This is a phase II clinical trial which will be conducted in 2 stages: Stage 1 is a safety and dose-finding stage involving 4 groups of 5 patients. Stage 2 is a randomised, controlled, open-label trial of alteplase.
Planned sample size	Stage 1: 20 participants Stage 2: 50 participants
Eligibility Criteria **Inclusion criteria**	1. Diagnosis of acute respiratory failure (ARF) with severity graded by the arterial oxygen partial pressure to inspired fraction of oxygen ratio (P_aO_2/F_iO_2) as per the Berlin definition: acute onset of hypoxemia with an arterial partial pressure of oxygen (P_aO_2) to inspired fraction of oxygen (F_iO_2) ratio of less than or equal to 300 mmHg with positive end expiratory pressure (PEEP) of 5 cm H_2O or greater. The ARF may be of primary pulmonary infectious or extrapulmonary infectious aetiology. 2. Requiring admission to the Intensive Care Unit (ICU) 3. Aged 18 – 75 years of age 4. Procoagulant profile on ClotPro Fib-test **+/-** EX-test – above normal range for A10 and/or MCF at 30 mins 5. Lysis Time on ClotPro TPA-TEST ClotPro equal to or greater than 365 seconds
Exclusion criteria	1. Platelet count < 125 x 10^9/L or a reduction in platelet count of 50 x 10^9/L or more within last 24 hours. 2. Body weight < 60 kg 3. Structural intracranial disease e.g. arterio-venous malformation or aneurysm 4. Previous intracranial haemorrhage

	5. Ischaemic stroke within 3 months 6. Primary and/or metastatic solid tumours 7. Traumatic cardiopulmonary resuscitation 8. Hypoxaemia from traumatic lung injury 9. Active or recent bleeding 10. Surgery, trauma or invasive procedure within 4 weeks – if after this period, consult surgeon first. 11. Systolic BP > 180 mm Hg 12. Diastolic BP > 100 mm Hg 13. Pericarditis or pericardial fluid 14. Diabetic retinopathy 15. Currently menstruating 16. Pregnancy – (βHCG to be performed if of child-bearing age) 17. Liver failure (known severe liver disease or an alanine aminotransferase or an aspartate aminotransferase level that is 5 times the upper limit of normal) 18. Kidney failure (estimated Glomerular Filtration Rate (eGFR ≤30 mL/hr or receiving renal replacement therapy) 19. Use of therapeutic anticoagulation or platelet antagonists (see Appendix 1) 20. Not for active treatment 21. Unlikely to survive until the day after tomorrow
Study intervention	The study will be conducted in two stages: the first, a safety and dose-finding stage of escalating alteplase doses, and the second a randomised, controlled, open-label, Phase II clinical trial comparing VET-guided alteplase treatment with the dose tailored according to the patients lysis time on VET + standard care, with standard care alone in the same patient disease group.
Study duration	Approximately 24 months

2.0 BACKGROUND - ARF

Acute respiratory failure (ARF) most commonly develops in response to infection or a systemic inflammatory state. A recent and prominent example of infectious ARF is caused by COVID-19 and is characterised by progressive respiratory failure and a distinct coagulopathy. A study of 184 COVID-19 patients admitted to Intensive Care revealed a thrombotic complication rate of 31% [1]. Autopsies conducted on COVID-19 patients have demonstrated widespread thrombosis throughout the lungs [2, 3], and a deep vein thrombosis rate of 58% with pulmonary embolism the cause of death in 30% [4]. Elevated D-dimer levels, a byproduct of clot breakdown, is associated with poor patient outcomes in COVID-19 patients, with some critically-ill patients have D-dimer levels well above the detection limits of standard laboratory testing [5, 6].

Other non-COVID-19 infectious forms of ARF are also associated with a pro-coagulant phenotype [7, 8]. In the 1980's several pulmonary angiography studies were undertaken in ARF patients demonstrating the presence of multiple pulmonary vessel filling defects that associated with the severity of disease and patient outcome [9-11], and that resolved following the administration of the fibrinolytics, streptokinase and urokinase [12]. An early phase I study reported improved oxygenation in patients with severe ARF following administration of plasminogen activators [13]. The rationale for

fibrinolytics in ARF has been published previously [[14, 15] and is supported by a meta-analysis of preclinical studies [16].

In both non-COVID-19 and COVID-19 associated ARF, defective natural fibrinolysis has been demonstrated [17-23]. If fibrinolysis (clot breakdown) becomes compromised, as is seen in ARF patients, D-Dimer levels may not reflect the full extent of thrombosis present, thus they are not an accurate indicator of the severity of disease. Standard coagulation tests are also unhelpful in these patients as they cannot identify a hypercoagulable state nor assess fibrinolysis.

2.1 Viscoelastic Testing (VET)

Viscoelastic testing (VET) is a proven point-of-care technology approved by the TGA for the global assessment of whole blood coagulation. In contrast to standard coagulation testing, VET is able to provide insight into procoagulant phenotypes, fibrinolysis, the extrinsic and intrinsic coagulation pathways, fibrin clot formation, platelet contribution to clot formation, tissue plasminogen activator sensitivity and more. As a point-of-care device it has a rapid turn-around time of 10 minutes for initial information on clotting status.

VET has improved internationally the anticoagulation and blood product transfusion practices in cardiac bypass surgery, trauma and liver failure [24]. It is already used extensively to guide patient care in the above-mentioned indications at the Liverpool Hospital Intensive Care Unit.

2.2 VET in ARF

Numerous studies have demonstrated that VET is sufficiently sensitive to detect the coagulopathies associated with ARF, with several parameters associating with the severity of disease [23, 25]. Studies in COVID-19 patients consistently show increased clot dimensions and strength on Ex-Test, as depicted in Figure 1, with reduced clot lysis upon tPA challenge, TPA-Test, as depicted in Figure 2. **Table 1** summarises the results of TPA-tests in COVID-19 patients.

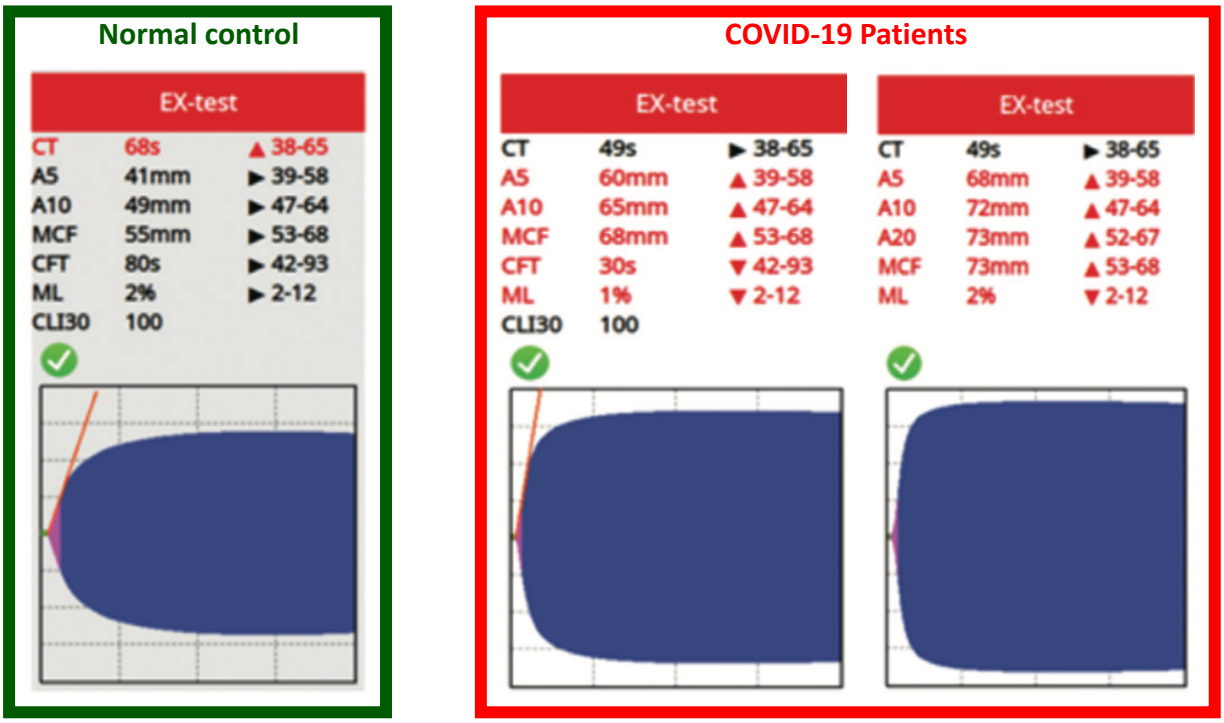

Figure 1: ClotPro results demonstrating hypercoagulation in COVID-19 patients
Upon addition of tissue factor to blood (Ex-Test), clot formation occurs and key parameters can be measured such as time to onset of clotting (CT), amplitude of the clot at 5 (A5) and 10 (A10) min following the onset of clotting, and maximum clot firmness (MCF) [26].

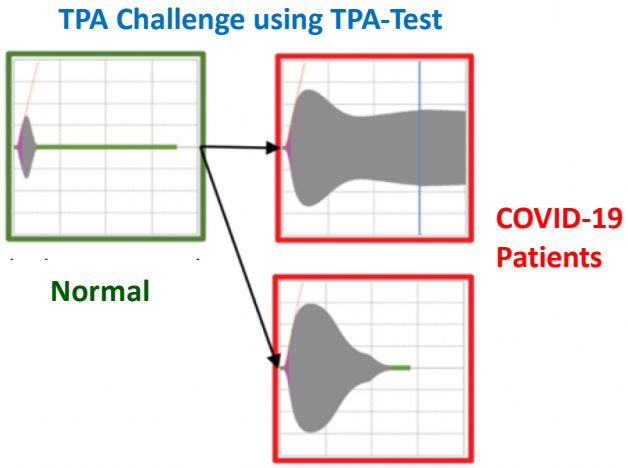

Figure 2: ClotPro results demonstrating hypofibrinolysis in COVID-19 patients
The TPA-Test involves the addition of tPA and tissue factor to the blood sample then measuring the clotting response. In healthy subjects (top left panel), the clot is lysed by tPA as it forms, whereas in COVID-19 patients, clot lysis occurs slowly (bottom right panel) or not at all (top right panel). Lysis Time (LT) is a measure of the time taken for the maximum clot firmness to decrease by 50% [23].

Table 1: TPA-Test Lysis Time (seconds) in COVID-19 patients and healthy controls

COVID-19 patients	Healthy Controls	Difference (95% CI), *p*-value	Reference
530 +/- SD 327, **n=27** Critically ill	211 +/- SD 80, **n=12**	319 (182,456), p<0.001	[20]
400 +/- SD 93, **n=7** Critically ill	201 +/- SD 63, **n=15**	199 (111, 287), p<0.001	[21]
368 +/- SD 125, **n=7** Non-critically ill	201 +/- SD 63, **n=15** (same cohort as above)	167 (50, 284), p<0.001	[21]
508 (365 – 827), **n=20** (25^{th} – 75^{th} percentile) Critically ill	210 (186-261), **n=60** (25^{th} – 75^{th} percentile)	298 (151, 445), p<0.001	[22]

2.3 Plasma tPA and PAI-1 levels in ARF

Plasminogen activator inhibitor-1 (PAI-1) is released from platelets, innate immune cells, endothelium and hepatocytes and is elevated in infection [27]. Circulating PAI-1 was found to be elevated in severe pneumonia and non-COVID-19 ARF, as a result of release from alveolar macrophages, while endothelial cells produced lower levels of tissue plasminogen activator (tPA), thus creating a pro-thrombotic state [19]. In ARF injury, PAI-1 levels in pulmonary fluid was found to correlate with mortality [28].

VET and non-VET studies have demonstrated a resistance of clots from COVID-19 patients to the fibrinolytic effects of added tissue plasminogen activator (tPA) reflecting the presence of high levels of PAI-1 [23, 26]. A study examining tPA and PAI-1 levels in COVID-19 patients demonstrated that high levels of both these factors were associated with severe disease and death. While some patients had high tPA levels thus a hyperfibrinolytic bleeding disease phenotype, others had high PAI-1 levels and a hypofibrinolytic thrombotic disease phenotype [26].

These data demonstrate that coagulopathy occurs with all forms of ARF.A personalised approach to the management of coagulopathic ARF patients is essential and this can only be achieved in a clinically-relevant timeframe with the use of VET.

2.4 tPA treatment for myocardial infarction, stroke and pulmonary embolism

tPA is standard treatment for myocardial infarction and stroke and has demonstrated substantial benefit in preserving functional tissue in the heart and brain through the lysis of blood clots blocking arteries to these essential organs [29, 30]. The most commonly used protocol in both situations is to infuse 0.9 mg/kg (maximum dose 90 mg) intravenously over 1 hr with 10% of the total dose administered as an initial bolus over 1 minute [31].

Patients with pulmonary embolism also benefit significantly from treatment with thrombolytic therapy including tPA [32]. A low-dose (50mg infusion over 2 hr) was found to be equally effective as a high-dose (100 mg infusion over 2 hr) with a reduced incidence of bleeding in a meta-analysis of trials [33].

2.5 tPA treatment in ARF

Unlike myocardial infarction, stroke and PE, infectious ARF is associated with a disseminated and ongoing coagulopathy due to severe infection and the immune system response. Several animal models of ARF [16, 34, 35] and small studies in non-COVID-19 ARF patients using fibrinolytic therapy have demonstrated clearance of microthrombii from within the pulmonary vasculature and improved lung function [12, 36, 37]. The reason that fibrinolytic therapy has not been adopted as standard treatment for ARF is the potential bleeding risk [38], despite no bleeding episodes occurring in a study of 18 ARF patients [37]. A keen but cautious interest remains in the use of tPA for non-COVID-19 ARF.

In a small series of COVID-19 patients with ARF, 2 of 3 patients received temporary benefit, in the form of improved lung function, from the administration of tPA 25 mg as a i.v. bolus then a further 25 mg over 24 hr as an i.v. infusion without any bleeding complications occurring [39]. In the US, tPA was considered an option for COVID-19 in desperate times when ICU- and ventilator beds were exhausted [40]. Given the pathophysiology of COVID-19 disease, there is significant interest in the use of thrombolytics, however, the selection of patients most likely to benefit from this treatment based upon standard laboratory testing is considered problematic [40, 41].

VET, more specifically ClotPro, has been shown capable of distinguishing between the hyper- and hypolytic and hyper- and hypocoagulant phenotypes in COVID-19, and therefore identifying patients who will most likely benefit from tPA treatment. Of equal importance, VET will also identify patients who have a bleeding risk and, hence, who should not receive tPA treatment. In terms of optimising the treatment effect and minimising bleeding risk, ClotPro provides the ability to quantify the response to tPA therapy thus permitting dose titration.

2.6 Current Clinical Trials with tPA in COVID-19 patients

The following is a summary of clinical trials that are using thrombolytics in COVID-19 patients and that are entered into the international ClinicalTrials.gov website. There are currently no studies registered using thrombolytics in non-COVID-19 ARF.

In situ thrombolysis with tPA and inflow perfusion analysis in patient with severe COVID-19 infection.
This was a compassionate study undertaken in Mexico in 15 patients who had tPA injected directly into their pulmonary arteries under fluoroscopic guidance and an assessment of efficacy made on the distribution of contrast before and after the procedure.

This trial is complete but no results are provided.

A study to test whether different doses of Alteplase (tPA) help people with severe breathing problems because of COVID-19 (TRISTARDS)
This Boehringer Ingelheim sponsored study being conducted in Europe is recruiting and is described as administering a low-dose or high-dose alteplase infusion for 5 days on top of standard care. The dose details are not provided. An interim analysis of Phase IIb of this study, that included 62 patients randomised to receive 1 of 2 dosing regimes of alteplase for up to 5 days in combination with standard of care or standard of care alone, demonstrated favourable safety and efficacy data (https://www.boehringer-ingelheim.com/press-release/bi-focuses-covid-19-clinical-research-alteplase).

Tenecteplase in patients with COVID-19
This placebo controlled study to be conducted in Israel is determining the efficacy of the longer-acting thrombolytic agent, Tenecteplase, in COVID-19 patients with respiratory failure. There will be 2 active treatment groups who will receive low- (0.25 mg/kg) or high-dose (0.5 mg/kg) Tenecteplase as an i.v. bolus in combination with heparin.

Fibrinolytic therapy to treat ARF in the setting of COVID-19 infection
This randomised controlled study to be conducted in the US will have 2 active treatment groups. The first group will receive tPA treatment 50 mg administered as an i.v. bolus (10 mg) followed by the remaining 40 mg given as in infusion over 2 hr. Immediately afterwards patients will commence on an i.v. heparin infusion. Patients may receive an additional 50 mg between 24 and 36 hr if an improvement in P_aO_2/F_iO_2 is seen. The second group will receive a tPA infusion of 2 mg/hr for a 24 hr period after the initial bolus and 2 hr infusion. Following the 24 hr infusion of tPA, patients are commenced on an i.v. heparin infusion. This study is active but not recruiting [42, 43].

2.7 How will this study contribute to the field?

The last 3 studies summarised above will be recruiting patients based upon their D-dimer levels +/- evidence of PE on CTPA +/- the level of respiratory dysfunction. D-dimers levels reflect clot breakdown, or fibrinolysis. When the system is overwhelmed however, as is seen in ARF patients, fibrinolysis is compromised thus the level of D-dimers does not necessarily reflect the levels of thrombosis. Critically-ill ARF patients are not routinely screened for pulmonary emboli as this would delay the commencement of life-saving treatment. The degree of respiratory distress is not an accurate measure of the need for tPA administration as it may be due to the bacterial/viral load, immune response, and/or pre-existing lung conditions as well as thrombotic processes.

This study will be recruiting patients admitted to the ICU with ARF based upon their VET results, specifically, the demonstration of a pro-coagulant profile on the FIB-Test +/- EX-Test, and delayed lysis on the TPA-Test. In the TPA-Test, the blood sample is supplemented with tPA. Delayed lysis provides further evidence of a pro-coagulant phenotype and the length of time required for lysis to occur provides a measure of the severity of fibrinolysis shutdown. We will, therefore, be selecting patients who may benefit from tPA treatment using a direct measure in real-time of coagulation and fibrinolysis. In addition to this targeted patient selection process, VET will be employed to closely monitor the response of each patient to tPA treatment and the dose tailored according to this response. This personalised approach to tPA therapy is similar to the management of i.v. heparin where the dose is dictated by aPTT levels, whereas in this case, Lysis Time will be dictating tPA dosing. This will significantly increase the likelihood of a therapeutic dose being delivered and decrease the risk of bleeding. Such an approach to tPA treatment has not been previously undertaken, thus this study will potentially provide a new protocol for improved tPA administration. It will also add significant insight into the kinetics of the reversal of fibrinolysis imbalance in severe infection through tPA supplementation.

3.0 STUDY AIMS & HYPOTHESES

The aims of this study are to:

(i) *Stage 1:* establish the safety and dose range of VET-guided alteplase treatment in ARF patients at high risk of thrombosis.

(ii) *Stage 2:* determine whether the administration of alteplase to ARF patients at high risk of thrombosis normalises fibrinolysis and improves organ function.

The hypothesis underpinning the study aims is that the administration of alteplase will restore the tPA/PAI-1 balance allowing fibrinolytic activity to occur normally, thus preventing thrombosis-induced ischaemia of organs and poor patient outcomes.

4.0 PARTICIPATING SITES

The study will be conducted in the Intensive Care Units of Liverpool and Bankstown Hospitals, NSW.

5.0 STUDY DESIGN

The study will be conducted in 2 stages:

Stage 1 is a safety and dose-finding stage involving 4 groups of 5 patients.
Stage 2 is a Phase II, randomised, controlled, open-label trial.

6.0 EXPECTED DURATION

It is anticipated this study will commence in January 2022 and be completed in 2023.

7.0 PATIENT POPULATION

Patients who are admitted to the Intensive Care Units at Liverpool and Bankstown Hospitals will undergo routine viscoelastic testing for a broad assessment of coagulation status. The ClotPro machine will be used for this study as it offers a TPA-Test to assess fibrinolysis along with the EX-Test and Fib-Test. Those patients who are identified as being hypercoagulant and hypofibrinolytic on VET testing will be considered suitable for further screening.

8.0 ELIGIBILITY CRITERIA

8.1 Inclusion Criteria

1. Diagnosis of acute respiratory failure (ARF) with severity graded by the arterial oxygen partial pressure to inspired fraction of oxygen ratio (P_aO_2/F_iO_2) as per the Berlin definition: acute onset of hypoxemia with an arterial partial pressure of oxygen (P_aO_2) to inspired fraction of oxygen (F_iO_2) ratio of less than or equal to 300 mmHg with positive end expiratory pressure (PEEP) of 5 cm H_2O or greater. The ARF may be of primary pulmonary infectious or extrapulmonary infectious aetiology.

2. Requiring admission to Intensive Care
3. Aged 18 – 75 years of age
4. Procoagulant profile on ClotPro Fib-test +/- EX-test – above normal range for A10 and/or MCF at 30 mins run time
5. Lysis Time on ClotPro TPA-TEST ClotPro *equal to or greater than* ***365 seconds***

8.2 Exclusion Criteria

1. Platelet count <125 x 10^9/L or a reduction in platelet count of 50 x 10^9/L or more in the last 24 hours.
2. Body weight < 60 kg
3. Structural intracranial disease e.g. arterio-venous malformation or aneurysm
4. Previous intracranial haemorrhage
5. Ischaemic stroke within 3 months
6. Primary and/or metastatic solid tumours
7. Traumatic cardiopulmonary resuscitation
8. Hypoxaemia from traumatic lung injury
9. Active or recent bleeding
10. Surgery, trauma or invasive procedure within 4 weeks – if after this period, consult surgeon first.
11. Systolic BP > 180 mm Hg
12. Diastolic BP > 100 mm Hg
13. Pericarditis or pericardial fluid

14. Diabetic retinopathy
15. Currently menstruating
16. Pregnancy – (βHCG to be performed if of child-bearing age)
17. Liver failure (known severe liver disease or an alanine aminotransferase or an aspartate aminotransferase level that is 5 times the upper limit of normal)
18. Kidney failure (estimated Glomerular Filtration Rate (eGFR ≤30 mL/hr or receiving renal replacement therapy)
19. Use of therapeutic anticoagulation or platelet antagonists (see Appendix 1)
20. Not for active treatment
21. Unlikely to survive until the day after tomorrow

9.0 STUDY OUTLINE & PROCEDURES

This trial will consist of 2 stages, the first a safety and dose-finding stage, and the second a randomised, controlled trial of VET-guided alteplase treatment + standard care versus standard care alone.

9.1 Stage 1: Safety & Alteplase Dose-Finding

Upon admission to ICU, all ARF patients will have a 2.7 mL blood sample collected to enable baseline VET analysis which would include: EX-Test, plasminogen-test, Fib-Test, TPA-Test run over 30 minutes. This aspect of the trial will not require consent as VET is part of routine ICU assessment in patients with a potential coagulopathy. Weight will be estimated and a urinalysis via dipstick will be undertaken as per standard care to confirm the absence of haematuria.

The VET results will be used to identify patients who are hypercoagulopathic (Ex-Test parameters) and hypofibrinolytic (Lysis Time on TPA-Test of *equal to or greater than 365 seconds*) thus at high risk of organ damage due to micro- and macro-thromboembolism.

Group 1 will consist of 5 participants who will receive the following treatment after being screened for eligibility and consented to the study:

- alteplase treatment will commence with an i.v. infusion of 25 mg over 2 hr, then an i.v. infusion of 1 mg /hr for 22 hr*
- VET will be repeated at 3, 6, 12, 18 and 24 hr post-infusion commencement, and 1 hr post cessation. Alteplase is rapidly cleared by the liver with a half-life of 4-10 minutes thus a coagulation profile will be obtained following 24 hr of, and then in the absence of alteplase administration.

* Total dose over 24 hr = 47 mg.

Group 2 will consist of 5 participants who will receive the following treatment after being screened for eligibility and consented to the study:

- alteplase treatment commenced with an i.v. infusion of 50 mg over 2 hr, then an infusion of 2 mg/hr over 22 hr*
- VET will be repeated at 3, 6, 12, 18 and 24 hr post-infusion commencement, and 1 hr post cessation.

* Total dose over 24 hr = 94 mg.

Depending upon the results obtained in the first 2 groups of patients:

Group 3 will consist of 5 participants who will be selected *based upon a Lysis Time on the TPA-test of 426 – 500 seconds.* Following screening for eligibility and consent to the study they will receive:

- alteplase treatment commenced with an i.v. infusion of 50 mg over 2 hours, then an infusion of 4 mg/hr mg over 22 hr*
- VET will be repeated at 3, 6, 12, 18 and 24 hr post-infusion commencement, and 1 hr post cessation.

* Total dose over 24 hr = 138 mg

Group 4 will consist of 5 participants who will be selected *based upon a Lysis Time on the TPA-test of >500 seconds.* Following screening for eligibility and consent to the study they will receive:

- alteplase treatment commenced with an i.v. infusion of 50 mg over 2 hours, then an infusion of 4 mg/hr over 22 hr*
- VET will be repeated at 3, 6, 12, 18 and 24 hr post-infusion commencement, and 1 hr post cessation
- if VET results at 12 hr have not improved from baseline, a 25 mg alteplase i.v. bolus will be administered and the infusion and VET monitoring continued.

* Total dose over 24 hr = 138 +/- 25 mg.

***In all of the above dosing schedules, the maximum alteplase dose administered within the first 2 hours is less than the dose administered *within 1 hr* for myocardial infarction or stroke under standard tPA treatment protocols (0.9 mg/kg with a maximum of 90 mg).

*********The half-life of alteplase is very short being 4-10 minutes.***

Treatment Stopping Rules:
Alteplase treatment will be ceased should any of the following events occur:

- Lysis Time on repeat TPA-Test is **less than 250 seconds**
- Grade 2+ bleeding event (CTCAE grading system, see below: Reporting of Serious Adverse Events)
- Reduction in platelet count to <100 x 10^9/L.
- Decision by the attending Intensive Care Specialist
- All participants should be continued on venous thromboembolism (VTE) **prophylaxis** as per standard care (s.c. low-molecular weight heparin (LMWH) / unfractionated heparin (UF) throughout).

9.2 Stage 2: Phase II Randomised, Controlled, Open-Label Trial

Patients randomised to receive alteplase treatment
The following protocol, including alteplase doses and timing of repeat VET testing, will be reconsidered in the light of results obtained from Stage 1.

Upon admission to ICU, all ARF patients will have a 2.7 mL blood sample collected to enable baseline VET analysis which would include: EX-Test, plasminogen-test, Fib-Test, TPA-Test run over 30 minutes. This aspect of the trial will not require consent as VET is part of routine ICU assessment in patients with a potential coagulopathy. Weight will be estimated and urinalysis via dipstick will also be undertaken as per standard care to confirm the absence of haematuria.

The VET results would be used to identify patients who are hypercoagulopathic and hypofibrinolytic and thus, at high risk of organ damage due to micro- and macro-thromboembolism. Patients will be consented and randomised to receive standard anticoagulation or VET-guided alteplase treatment.

Participants randomised to the alteplase arm will be commenced on alteplase treatment according to the lysis time on TPA-Test as per the following treatment chart:

Chart 1: Alteplase Treatment Commencement Guide

Lysis Time on TPA-Test of 365 – 425 seconds **50 mg** i.v. infusion over 2 hr, then **2 mg/hr** i.v. infusion
Lysis Time on TPA-Test of 426 – 500 seconds **50 mg** i.v. infusion over 2 hr, then **4 mg/hr** i.v. infusion
Lysis Time on TPA-Test of greater than 500 seconds **50 mg** i.v. infusion over **1** hr, then **4 mg/hr** i.v. infusion over 24 hr

*Every **6 hours post-infusion commencement**,* VET will be repeated on all patients:

- If the Lysis Time is **less than 250 seconds** the alteplase infusion will be ceased and 6 hrly VET monitoring **continued****.
- If the Lysis Time is **250 - 364 seconds** the alteplase infusion will be reduced to 1mg/hr.
- If the Lysis Time is between **365 – 425 seconds,** the alteplase infusion will remain at, or be changed to 2 mg / hr.
- If the Lysis Time is between **426 and 500 seconds,** the alteplase infusion will remain at, or be changed to 4 mg / hr.
- If the Lysis Time is **greater than 500 seconds**, an alteplase bolus of **25 mg** will be administered i.v. over 1 min and the i.v. alteplase infusion continued at 4 mg / hr.

***** alteplase **treatment will continue for a maximum of 72 hours following the study start time with a minimum of 6 hrly VET monitoring throughout this period***

****If within the 72 hr treatment period, the Lysis Time increases above 365 seconds in a patient where the** alteplase **was previously ceased,** alteplase **should be recommenced at 2 mg/hr.**

Treatment Stopping Rules:
Alteplase treatment will be permanently ceased should any of the following events occur:

- Reduction in platelet count to <100 x 10^9/L.
- Grade 2+ bleeding event (CTCAE grading system, see below: Reporting of Serious Adverse Events)
- 72 hours of alteplase treatment has been administered
- Decision by the attending Intensive Care Specialist

All participants should continue VTE prophylaxis throughout the alteplase treatment period.

VET will be repeated on all participants 24 hr following cessation of the alteplase treatment period.

Participants Randomised to the Standard Control Arm
VET will be conducted every 6 hours for a 72 hr period then again at 96 hr following consent.

10.0 STUDY OUTCOME MEASURES

10.1 Primary Outcome Measure

Changes in Lysis Time on TPA-Test from baseline to 72 hours

10.2 Secondary Outcome Measures

Changes in all other VET parameters from baseline to 72 hours
Incidence of thromboembolism to 30 days or hospital discharge, whichever occurs first
Incidence of bleeding requiring medical intervention to 5 days post alteplase cessation
Changes in organ function – lung, liver, kidneys, haematology (P_aO_2/F_iO_2 ratio and SOFA scores) from baseline to 72 hours

11.0 STATISTICAL CONSIDERATIONS

Results will be reported using descriptive statistics for normally and non-normally distributed data. Comparison between groups will be performed using non-paired t-testing as appropriate for normally (Student t-test) or non-normally (Mann-Whitney U-test) distributed data. Repeated measurements will be reported using mixed factor analysis of variance (ANOVA) with time as within-subjects factor and group (tPA dose or tPA vs. standard care alone) as between-subjects factor.

In Stage 2 of the study, participants will be randomised on a 1:1 ratio to receive alteplase treatment + standard care or standard care alone.

An interim analysis will occur once 50% of patients (12 in each arm) have been recruited to the study enabling the reassessment of safety and early detection of detriment, futility or benefit of alteplase treatment.

11.1 Sample Size and Power

Based on the primary outcome of interest, **a sample size of 25 study participants in each arm of the study**, will have a power of 0.99 and 0.94 to detect a 2-SD and 1-SD (125 seconds, cf. Table 1) difference in TPA-test lysis time between the intervention and control participants, respectively.

12.0 CONSENT PROCESS

The consent process for this clinical trial will be tailored to the physical status of the patient. Additionally, the patients may be in isolation if infected with COVID-19 in which case the ICU specialists will consent in one of the manners outlined below:

1. Patients in ICU not on a ventilator or heavily sedated: The ICU specialist will provide a copy of the Participant Information and Consent Form (PICF) to the patient and discuss the clinical trial with them. The patient will be given the opportunity to ask questions and consider participating in the study. They will be advised that participation is voluntary and they can withdraw at any time. If the patient is willing to participate in the clinical trial the ICU Specialist and the patient will sign the consent form. If the patient has COVID-19, a photo of the consent form will be taken as the PICF cannot be removed from the isolation room. A copy of this will be sent to the patient and filed in their medical records.

2. Patients in ICU ventilated or heavily sedated with a responsible person present in the hospital: The ICU specialist will provide a copy of the PICF to the responsible person and discuss the clinical trial with them. The responsible person will be given the opportunity to ask questions and consider whether the patient should participate. The responsible person will be advised that participation is voluntary, the patient can be withdrawn at any time and that if they decide not to participate they will receive alternative treatments. If the responsible person is willing to consent to the patient being involved in the clinical trial the PICF will be signed. A copy will be provided to the responsible person. When the patient has sufficiently recovered the PICF will be provided to the patient and if the patient is willing to participate in the clinical trial the ICU Specialist and the patient will sign the consent form. In the event the patient is not willing to consent, the study staff and Sponsor will be notified that the data cannot be used.

3. Patients in ICU ventilated or heavily sedated with a responsible person not available to meet with the ICU Specialist face to face: The ICU specialist will contact the responsible person via telephone and discuss the clinical trial with them. A copy of the PICF will be emailed to the responsible person and s/he will be given the opportunity to ask questions and consider whether the patient should participate. The responsible person will be advised that participation is voluntary, the patient can be withdrawn at any time and that if they decide not to participate they will receive alternative treatments. The ICU Specialist will arrange a video conference with the responsible person once they have had time to review the information and via video conference the responsible person is willing to consent to the patient being involved in the clinical trial. This video conference will be recorded and used as oral consent and filed in the patient's medical records and the Investigator Site File. A copy will be provided to the responsible person. When the patient has sufficiently recovered the PICF will be provided to the patient and if the patient is willing to participate in the clinical trial the ICU Specialist and the patient will sign the consent form. In the event the patient is not willing to consent, the study staff and Sponsor will be notified that the data cannot be used.

13.0 RANDOMISATION PROCESS

A computer generated randomisation list will be used to assign patients to standard care or alteplase treatment.

14.0 ETHICAL CONSIDERATIONS

While alteplase administration is an experimental treatment approach in ARF patients, the logic is sound given the pathophysiology and the evidence of a significant pro-thrombotic risk associated with COVID-19 and other lung infections, and there is evidence of benefit from animal studies and small-scale clinical trials.

Alteplase is not an experimental medicine having been used for several decades in patients suffering myocardial infarction, stroke or pulmonary embolism. For myocardial infarction and stroke, the amount of alteplase used is higher and delivered over a shorter time frame than what is proposed in this study which has been designed to be more in line with protocols for pulmonary embolism.

This study is taking advantage of VET technology to increase the safety and efficacy of alteplase treatment in this population. VET will ensure that patients at greatest risk of thrombosis are selected for screening, and will eliminate those patients at risk of bleeding complications. Additionally, ongoing VET testing throughout the alteplase treatment period will ensure an optimal dose is delivered thus minimising the risk of over-dosing with potential bleeding complications, but also reducing the likelihood of under-dosing and having no therapeutic effect.

14.1 Ethics Review

The final study protocol, including the final version of the ICF, must be approved or given a favourable opinion in writing by an HREC as appropriate.

The PI is responsible for informing the HREC of any amendment to the Protocol in accordance with local requirements. In addition, the HREC must approve all advertising used to recruit participants for the study. The protocol must be re-approved by the HREC upon receipt of amendments and annually, as local regulations require.

The PI is also responsible for providing the HREC with reports of any reportable serious adverse drug reactions from any other study conducted with the study drug (active).

Progress reports and notifications of serious adverse drug reactions will be provided to the HREC according to local regulations and guidelines.

15.0 REPORTING of SERIOUS ADVERSE EVENTS and BLEEDING EVENTS

All SAEs or bleeding events Grade 3+ should be emailed to:
Prof Anders Aneman anders.aneman@health.nsw.gov.au *AND*
Dr Lucy Coupland l.coupland@unsw.edu.au **within 24 hr of occurrence**.

Definition of Serious Adverse Events

As eligible patients are critically-ill and requiring admission to Intensive Care, their condition prior to entering the study is already a serious event. Reporting of serious adverse events that occur in study participants will, therefore, be **serious adverse events that are considered *unrelated* to underlying disease progression in the opinion of the Investigator.**

15.1 Reporting of bleeding events

As bleeding is the most likely side-effect of alteplase treatment, ALL bleeding events Grade 3 or greater according the CTCAE (version 5.0) grading system, will be reported as an SAE *irrespective of whether it is considered related to study-treatment or not*.

Grade 1 events, those not requiring medical intervention, will *not be* reported as critically-ill patients have multiple intravenous/arterial access devices that ooze small amounts of blood at the insertion sites. Grade 2 events will be reported as an **Adverse Event.**

Grade 1	Minor bleeding, intervention not indicated – *will not be reported*
Grade 2	Moderate bleeding, intervention indicated
Grade 3	Transfusion indicated, invasive intervention indicated
Grade 4	Life-threatening consequences, urgent intervention indicated
Grade 5	Death

16.0 STUDY OVERSIGHT and INDEPENDENT MONITORING

The Study Committee, consisting of the Investigator Team, will meet upon the occurrence of all SAEs and Grade 3+ bleeding events; at the completion of each patient group on Stage 1; on a monthly basis; and following the interim analyses to review study progress and results.

An Independent Safety and Data Monitoring Committee (ISDMC) will also be established to oversee the study conduct. The ISDMC will consist of an Intensive Care Specialist, a Haematologist familiar with ClotPro and VET, a Respiratory Physician and a Senior Biostatistician. The ISDMC will be notified of all SAEs and Grade 3+ bleeding events within 24 hr of occurrence, and provided with a report *following each* of the 4 patient groups on Stage 1, and the interim analysis on Stage 2. The ISDMC will be responsible for determining whether it is safe and ethical for the study to continue, or whether the protocol requires amending following the occurrence of SAEs and bleeding events, and following each of the key study progress points. In Stage 1 of the study, recruitment to the next Patient Group will not occur until the ISDMC has provided approval to do so.

Members of the ISDMC are: Prof Anthony Delaney (Intensivist), Royal North Shore Hospital, Sydney University; Prof Christine Jenkins (Respiratory Physician), Concorde Hospital, The George Institute for Global Health; A/Prof Elizabeth Holliday (Medical Statistician), School of Medicine and Public Health, Newcastle University; Dr Philip Crispin (Haematologist), The Canberrra Hospital, Australian National University

17.0 STUDY PROCEDURE BENEFITS

There are significant potential benefits to participants enrolled on this study including the preservation of organ function and the avoidance of significant morbidity or death due to ARDS.

18.0 STUDY PROCEDURE RISKS

The risks associated with this study is the potential for bleeding complications associated with alteplase treatment. Bleeding may be mild, moderate or severe. This risk is being moderated through screening of participant risk prior to study enrolment, and by frequent VET monitoring throughout alteplase treatment. The volume of the alteplase infusion will be minimised to prevent fluid overload.

The amount of blood required for VET testing is minimal – 2.7 mL each time. This volume of blood at the frequency it is being collected will not impact upon an adult.

No other procedures are being conducted for the purposes of this study.

19.0 CONFIDENTIALITY & PRIVACY

19.1 Data Collection and Storage

Participants will be assigned a unique study number upon enrolment that will be used on all study documentation. A participant log will be maintained within the ICU Clinical Trials Unit of the Liverpool, Bankstown and Campbelltown Hospitals in a locked office so that re-identification can occur if required. Data provided to the Study Committee and the ISDMC will be de-identified but re-identifiable if required.

A RedCAP database will be established for the study and eCRFs designed to capture the **de-identified** demographic, disease severity, clinical, pathology and VET data that will be collected. The data will be backed up regularly, and 2 copies kept by the Principal Investigators (Aneman and Coupland), and accessible only by the Principal Investigators and Study Coordinators through password access.

19.2 Retention of Records

All source data, clinical records and laboratory data relating to the study will be archived for 15 years after the completion of the study. All data will be available for retrospective review or audit.

Source documents are original documents, data, and records from which the subject's eCRF data are obtained. These include, but are not limited to: hospital records, clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, angiograms, study drug accountability logs, and correspondence.

The Investigator and study staff are responsible for maintaining a comprehensive filing system of all study-related (essential) documentation. These include, but are not limited to: HREC correspondence, study drug accountability logs, and curricula vitae of all personnel participating in the study. These files must be suitable for inspection at any time by the Sponsor, monitor, and/or applicable regulatory authorities. All essential documentation should be retained by the institution for 15 years (as required in Australia).

No study document should be destroyed without prior written agreement between the Sponsor and the Investigator. If the Investigator wishes to assign the study records to another party or move them to another location, the PI must notify the Sponsor in writing of the new responsible person and/or the new location.

19.3 Disclosure and Confidentiality

The Investigator agrees to keep all information provided by the Sponsors in strict confidence and to request similar confidentiality from site staff and the local HREC. Study documents will be stored appropriately to ensure their confidentiality. The information provided by the Sponsors to the Investigator may not be disclosed to others without direct written authorization from the Sponsors, except to the extent necessary to obtain informed consent from participants who wish to participate in the study.

The Investigator must ensure that the participants anonymity is also maintained. Participants should only be identified by their initials and a study number on the eCRFs and other source documents. Other study-related documents (e.g., signed ICFs) should be kept in strict confidence by the Investigator.

20.0 STUDY TERMINATION

The study will be terminated should cumulative evidence indicate that alteplase treatment is detrimental. This decision will be made by the ISDMC.

Alternatively, should cumulative evidence suggest there is significant benefit to alteplase treatment, the study will be terminated, analysed and published as soon as possible. Again this decision will be made by the ISDMC.

21.0 FUNDING

A ClotPro machine has been donated to the Liverpool Hospital Intensive Care Unit for the purposes of this study by Haemoview Diagnostics Australia. Grant funds have been obtained to cover the purchase of a ClotPro machine, reagents and disposables for Bankstown Hospital ICU. The outcome of an additional grant application for the purchase of a ClotPro machine, reagent and consumables for Campbelltown Hospital ICU will be known in the near future.

The additional costs of the study at Liverpool Hospital will be covered by the Clinical Research Fund of the Liverpool Hospital Intensive Care Unit.

As SWSLHD is the Sponsor of this clinical trial, indemnity is provided by the Trust Management Fund as described in PD2011_006 Clinical Trials – Insurance and Indemnity.

22.0 RESEARCH OUTCOMES

Following the completion of this study, if alteplase treatment is demonstrated to restore fibrinolysis the Investigators will collaborate with the REMAP-CAP team at Monash University to undertake a larger trial with the power to determine whether improvements in organ function and patient outcomes also occur.

A Clinical Study Report will be prepared in accordance with ICH GCP requirements.

The results will be published expeditiously via the REMAP-CAP international network of Intensive Care clinicians, in a peer-reviewed international medical journal, and presented at medical meetings and conferences. If beneficial, it may also attract the attention of the media in which case a summary of the results would be provided in lay terms.

23.0 QUALITY CONTROL AND ASSURANCE

23.1 Compliance with Regulations

This study is being conducted under the TGA Clinical Trial Notification (CTN) Scheme

The study will be carried out in accordance with the current version of the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects), and with the NHMRC National

Statement on Ethical Conduct in Human Research 2007 (updated 2018). The conduct of the study will be in accordance with the Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice ICH E6 (R2), annotated with comments by the Australian Therapeutic Goods Administration (TGA; June 2018).

24.0 SPONSOR AND INVESTIGATOR OBLIGATIONS

24.1 Protocol Amendments

Neither the Investigator nor the Sponsors will modify or alter this protocol without the agreement of the other. All agreed protocol amendments will be clearly recorded on a protocol amendment form and will be signed and dated by the original protocol approving signatories. All protocol amendments will be submitted to the relevant institutional HREC for approval before implementation, as required by local regulations. The only exception will be when the amendment is necessary to eliminate an immediate hazard to the trial participants. In this case, the necessary action will be taken first, with the relevant protocol amendment following shortly thereafter.

24.2 Protocol Deviations

Should any protocol deviation occur, they must be reported to the Sponsor as soon as is reasonably practical. The deviation and the reason for its occurrence must be documented, reported to the relevant HREC (if required) and included in the CSR.

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26.0 Appendix 1: Anticoagulant and Platelet-Antagonists

Continued treatment with the following drugs, or others with similar actions, render the patient ineligible for this study:

Abciximab
Apixaban
Aspirin
Aspirin + Clopidogrel
Clopidogrel
Dabigatran
Eptifibatide
Non-steroidal anti-inflammatory drugs (NSAIDS)
Persantin
Rivaroxaban
Ticagrelor
Tirofiban
Warfarin